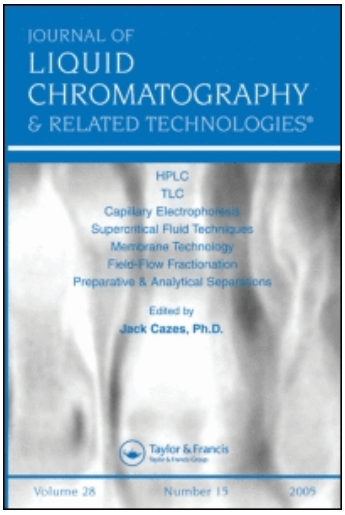

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Variables Influencing the Direct Determination of Haloacetic Acids in Water by Reversed-Phase Ion-Pair Chromatography with Indirect UV Detection

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VARIABLES INFLUENCING THE DIRECT DETERMINATION OF HALOACETIC ACIDS IN WATER BY REVERSED-PHASE ION-PAIR CHROMATOGRAPHY WITH INDIRECT UV DETECTION

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ABSTRACT

A reversed-phase ion-pair chromatographic system for the direct determination of haloacetic acids in water has been optimized utilizing indirect photometric detection. Acetic, chloroacetic, bromoacetic, iodoacetic, dichloroacetic and dibromoacetic acids were used to characterize the chromatographic system. The effect of temperature on retention time shows a non linear van't Hoff behavior indicating a change in the mechanism of retention at about 30°C. Above 30°C, retention times decreased proportionally to increases in the temperature of the column. Separations are achieved in the pH range of 3.5 to 6.3 with an optimum at ca. pH 5.4. Increases in the concentrations of KH_2PO_4, 1-hexanesulfonate (competing ion) and acetonitrile result in proportional decreases in capacity factors with some selectivity variations depending on the analyte. Increases in the concentration of benzyltributylammonium ion (ion interaction reagent) resulted in increases in capacity factors with a usable range from 8 to 12 mM. Of the reversed

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phases studied, Spherisorb C-18 yielded the optimal results for the acids studied. Linear calibration curves for these acids were obtained utilizing indirect UV detection with detection limits as low as 2 parts per million.

INTRODUCTION

Studies involving the formation and distribution of haloacetic acids in aquatic environments continue to be important and challenging tasks (1). Currently established methods, including EPA Method 552, involve extraction of the acids into organic solvents followed by derivatization into methyl esters for analysis by gas chromatography (2). Derivatization steps are time consuming and many derivatization reagents including diazomethane, used in EPA method 552, are toxic, carcinogenic and explosive hazards. Therefore, direct methods for the analysis of haloacetic acids without the need for derivatization are desirable. Reversed phase ion pair chromatography (RP-IPC) with indirect detection can be used to simultaneously separate neutral, ionic, and ionizable compounds with or without chromophores. Mobile phases in RP-IPC generally include 5 to 500 mM of an ion interaction reagent (also called a pairing ion or counterion). The most commonly used ion interaction reagent is the tetrabutylammonium ion although many others have been used, including different tetraalkylammonium ions, alkylsulfonic acids and trialkylamines (3-5).

The technique of ultraviolet detection for non ultraviolet absorbing solutes was first described by Schill et. al. (6) and later by Small and Miller (7) who called it "indirect photometry". Indirect fluorescence detection has also been reported (8,9). The choice of the wavelength in indirect photometry depends on the optical density of the ultraviolet absorbing eluent. Values greater than 1.6 may produce excessive noise and lead to a poor signal to noise ratio. Values lower than 0.4 may produce a poor signal. In the range 0.4 to 1.6, electronic compensation of the eluent absorbance to zero is possible without excessive noise (10). It has been suggested that 0.5 is an adequate upper limit for absorbance (11). The ultraviolet-absorbing component in the mobile phase will be in equilibrium with the stationary phase until an injected solutes disrupts that equilibrium either by binding or displacement giving rise to a detector response. This response can be positive or negative and is dependent on the retention and charge of the solute relative to the absorbing pairing ion. It has been suggested that the detection sensitivity for compounds of low retention can be improved if the mobile phase contains two

ultraviolet-absorbing ions with opposite charges and hydrophobicities, but with both having absorptivities in the same wavelength range (12).

The present RP-IPC method has been optimized and the relative effects of variations in the concentration of the ion interaction reagent, the organic modifier, the competing ion, the pH, the ionic strength, and the temperature of the separation have been evaluated.

System peaks

In HPLC, a chromatogram may show more peaks than the number of solutes in the sample, particularly when the mobile phase contains more than one component. These peaks have been called extra peaks, ghost peaks, pseudo peaks (13), vacancy peaks (14) and induced peaks (15), among other terms. The term "system peaks" will be used in this paper to indicate peaks not directly attributed to the actual solutes in the sample. The number of mobile phase components seen as system peaks depends on factors including the response of each particular compound to the detector in use. System peaks contain information about the thermodynamic (i.e., retention) and kinetic (i.e., broadening) processes that occur in the column. Thus, they can lead not only to a better understanding of the chromatographic process, but also to a better evaluation of the nature and amount of the solutes in the sample injected (16).

The origin of the system peaks has been studied in some detail elsewhere (16-18). After the mobile phase has passed through the chromatographic column for a certain period of time, an equilibrium between the stationary phase and the mobile phase constituents is reached and maintained as long as the chromatographic system is not disturbed. If anything in the mobile phase is changed, or if a solute is injected into the column, this equilibrium is disrupted and the chromatographic system immediately responds and adjusts to a new equilibrium state. The resulting injection zone, therefore, contains not only the injected solutes but also some, or all, of the mobile phase components at different concentrations than they are in the bulk mobile phase. System peaks have, in a given chromatographic system, constant capacity factors irrespective of the sample injected. They can appear positive or negative to the detector base line and their areas can depend on the nature of the injected solutes (16).

Mechanisms of retention

Many names have been used to describe ion pair chromatography including soap chromatography, solvent generated ion exchange, dynamic ion exchange, hetaeric chromatography, solvophobic-ion chromatography, ion interaction chromatography and chromatography on sorbed ionic sites. The exact mechanism by which separation occurs in reversed phase ion pair chromatography is still a matter of debate although three main models are popular with none completely satisfactory for all aspects of the phenomenon. Each is considered more an extreme situation than a comprehensive theory (19-21). According to the 'Ion Pair Model' or 'Partition Model' (22,23), ion pair formation occurs in the mobile phase prior to the adsorption or partition of the ion interaction reagent into the stationary phase. This model explains with greater precision the situation when a non-bonded reversed column is used and the stationary phase behaves as a bulk liquid, but is not able to explain ion-pair interactions with chemically bonded reversed phase columns.

The 'Dynamic Ion Exchange Model' (23-25) most closely explains chromatography when bonded reversed columns are in the system. The ion interaction reagent is envisioned to be absorbed onto the stationary phase surface. Once absorbed, the ion interaction reagent behaves as a liquid ion exchanger producing ionic interactions between the ionized solute molecules and the counterions adsorptively bound to the stationary phase. Finally, according to the 'Ion Interaction Model', ion pair formation is not necessary nor is the classical ion exchange interaction required to produce separation and retention. The model explains the process on the basis of a electrical double layer formation at the stationary phase surface as a result of the dynamic adsorption of the ion interaction reagent (19,26). The ion interaction model was used to predict that ultraviolet absorbing lipophilic ions added to a reversed phase eluent would coelute with non ultraviolet absorbing sample molecules to facilitate their detection (27). Figure 1 presents a schematic of some of the possible interactions among the bonded phase (C-18), the ion interaction reagent (benzyltributylammonium (BTA)), the competing ion (1-hexanesulfonate) and one of the compounds studied in this work (dichloroacetic acid).

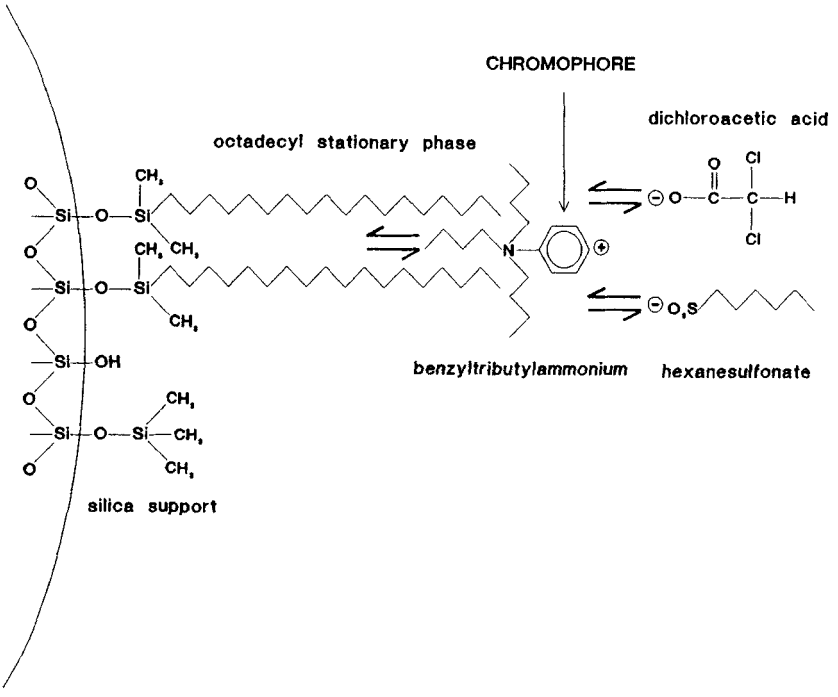

FIGURE 1 Schematic of several possible interactions among the bonded stationary phase (C18), the ion interaction reagent (benzyltributylammonium), the competing ion (1-hexanesulfonate) and one of the haloacetic acids studied (dichloroacetic acid).

MATERIALS

Benzyltributylammonium chloride, 1-hexanesulfonic acid sodium salt, 98% monobasic potassium phosphate, 98% bromoacetic acid, 98% iodoacetic acid, 99% dichloroacetic acid and 90% dibromoacetic acid were obtained from Aldrich Chemical Co. (Milwaukee, WI). HPLC grade water, HPLC grade acetonitrile and certified chloroacetic acid were obtained from Fisher Scientific (Fair Lawn, NJ). Analytical reagent grade glacial acetic acid was obtained from Mallinckrodt (St. Louis, Mo.). All chemicals were used as received, without further purification.

METHODS

Data was collected on a home-built HPLC system designed for portability consisting of a Scientific System Inc. (SSI) Model 300 LC pump (State College, PA), a SSI Model LP-21 LO-PULSE pulse controller, an Omega Engineering (Samford, CT) Type PSW Model 133 8835 gauge, and an E-Lab OMS TECH (Miami, FL) gradient controller and Chromatography System data manager. Sample injection was performed using a Valco (Houston, TX) injection valve with a 20μL sample loop. Temperature control was achieved with an Eppendorf (Madison, WI) TC-50 controller and CH-30 column heater. Detection was performed with an ISCO (Lincoln, NE) V4 UV-Visible absorption detector. The spectrum of the mobile phase was obtained with a Shimadzu (Columbia, MD) UV-2101PC UV-VIS scanning spectrophotometer. The fluorescence of the mobile phase was evaluated with a Perkin Elmer (Norwalk, CT) luminescence spectrometer Model LS50. The solutions were filtered using a filter degasser assembly from Aura Industries Inc. (Staten Island, NY). The columns compared were SGE (Austin, TX) glass-lined stainless steel of 4mmx25cm (IDxL) dimensions containing 5 μm particles with the following chemistry, pore sizes, surface areas and endcapping: Spherisorb C-18 (80Å, 220m²/g, fully); Phenyl (80Å, 220m²/g, partly); C-8 (80Å, 220m²/g, fully); Hypersil C-18 (120Å, 170m²/g, fully); Nucleosil C-18 (300Å, 190m²/g, fully); Nucleosil C-4 (300Å, 190m²/g, fully).

Mobile phases

The mobile phases and solutions were prepared using HPLC grade water. Eluents were filtered through a 0.45 μm Teflon membrane filter and thoroughly degassed with helium prior to use. The components of the mobile phase for this study were similar to that applied to the determination of inorganic anions (28), although using the same concentrations as those reported, resulted in no resolution of the haloacetic acids. Complete optimization procedures are described later. The mobile phases for the pH study were prepared so as to keep the ionic strength of all mobile phases constant by first increasing the mobile phase pH from 4.53 to 8.50 with sodium hydroxide or decreasing the pH to 2.30 with phosphoric acid,

followed by readjustment to the desired pH. The mobile phase showed three UV absorption maxima at 269, 263 and 258 nm. Table 1 summarizes the ultraviolet absorption of the studied acids at these wavelengths. Acetic, chloroacetic, bromoacetic and dichloroacetic acids do not absorb at all, dibromoacetic acid demonstrates minimal absorptivity in the 258-262 region and iodoacetic acid shows a maximum at 365, with a comparable absorptivity to the mobile phase when its concentration is 1318 parts per million (ppm).

Equilibration of system

Equilibration of an IPC system in some cases can take a very long time. Some authors recommend conditioning the system overnight (29); whereas, others suggest it is not necessary (10). Knox and Jurand (30,31) started with a mobile phase at a slightly higher concentration of the ion pair to reach the equilibrium faster. In the present study, equilibration was generally obtained within 1.5 to 2 hours. Once equilibrium has been obtained, to again reach the equilibrium

TABLE 1

Ultraviolet Absorptivities of the Ion Interaction Reagent and the Acids Studied

Compound	Concentration	Absorptivity at 269nm	263nm	258nm
Benzyltributylammonium	8 mM	2.59	2.78	2.66
Acetic acid	800 ppm	0.00	0.00	0.00
Chloroacetic acid	1511 ppm	0.00	0.00	0.00
Bromoacetic acid	858 ppm	0.00	0.00	0.00
Iodoacetic acid	1318 ppm	2.46	2.53	2.51
Dichloroacetic acid	1000 ppm	0.00	0.00	0.00
Dibromoacetic acid	1425 ppm	0.35	0.69	1.10

conditions after changing just one parameter, such as the temperature, generally takes less than 45 minutes. Regular replacement of frits and the pre-column packing also assisted in the rapid establishment of equilibrium and a stable baseline. The following have been observed when regular system maintenance was not performed: strange peak appearance (sometimes presenting a reproducible retention time), long time required for system stability, loss of resolution, erratic base line, unusual system peak patterns, sudden increases in signal (baseline) rising off scale. These problems were avoided by regular replacement of the pre-column packing material and the frits and filters before problems occurred.

Haloacetic acid standards

The toxicity, corrosiveness and hygroscopic properties of the haloacetic acids (see Table 2) make the handling of these compounds and the preparation of solutions at precise and reproducible concentrations difficult. To minimize these difficulties and to assure that the results were comparable, a single stock solution

TABLE 2

Physical and Chemical Properties of the Acids Studied

Acetic Acid	Formula Weight	Melting Point (°C)	Boiling Point (°C)	pKa (25°C)	Toxicity
Acetic	60.05	16	116-118	4.75	C
Chloro-	94.50	62-64	189	2.85	C, HT
Bromo-	138 95	49-51	208	2.69	C, L
Iodo-	185.95	77-79	-	3.18	C, T
Dichloro-	128.94	13.5	194	1.48	C, T
Dibromo-	217.84	48	195	1.39	C, T
Trichloro-	163.39	54-56	196	0.70	C, T

C = corrosive, HT = highly toxic, L = lachrymator, T = toxic

was carefully prepared with the following concentrations: acetic acid (800 ppm), chloroacetic acid (1511 ppm), bromoacetic acid (858 ppm), iodoacetic acid (1318 ppm), dichloroacetic acid (1000 ppm), and dibromoacetic acid (1425 ppm). All samples were then prepared from the same stock solution. The stock solution was regularly tested by looking for any changes in the UV spectra and regularly comparing ion pair chromatograms run under standard conditions to those run when the solution was first prepared.

RESULTS AND DISCUSSION

A mobile phase containing 8.22 mM benzyltributylammonium, 0.13 mM hexanesulfonate, 5.66 mM monobasic potassium phosphate, pH 5.2 and 11 per cent acetonitrile was able to produce the complete separation of the six acids from acetic acid to dibromoacetic acid in less than 20 minutes as shown in Figure 2. The chromatogram shows 6 positive peaks, one for each of the 6 acids injected preceeded by 5 distinct system peaks including a large negative peak which should correspond to the benzyltributylammonium ion. Trichloroacetic acid had a very long retention time (under the isocratic condition used in this study) which resulted in a severely broadened peak and poor detectability, and, therefore, was not included in the present optimization experiments. Our results are in agreement with prevoius workers who report positive UV absorbance (or fluorescence) when the sample and the ion interaction reagent are of opposite charge (as is in this case), and the sample components elute after the system peaks (32).

The capacity factors for all 7 acids listed in Table 2 was measured under isocratic conditions for the pKa comparison shown below, although gradient elution would be necessary to elute trichloroacetic acid with acceptable peak shape for quantitation. The capacity factors for the acids generally correlate with their acid dissociation constants as seen in Figure 3 for acetic acid through trichloroacetic acid. The pKa of the solute has been shown previously to have a significant effect on the retention of ionic solutes in ion-pair chromatography (33). Acids with higher Ka, at a given pH above their pKa, should have more anions available to interact with the ion interaction reagent and therefore should be more strongly retained than other similar acids with lower Ka values.

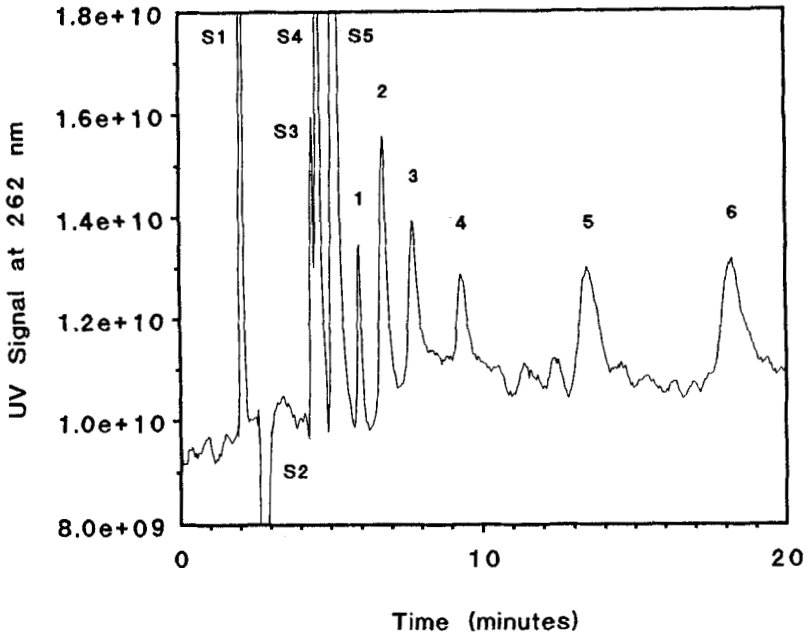

FIGURE 2 Typical isocratic separation of the haloacetic acids employing indirect UV detection. Peak identification: S1-S5 = system peaks; 1 = acetic; 2 = chloro-; 3 = bromo-; 4 = iodo-; 5 = dichloro-; 6 = dibromoacetic acid.

Effect of temperature

The effect of the temperature on the retention time of five haloacetic acids is shown in Figure 4 (complete data is listed in Table 3) using a Spherisorb C18 column. The system pressure fluctuated between 800 pounds per square inch for the higher temperature (58 centigrade degrees) and 1500 pounds per square inch for the lower temperature (22 centigrade degrees). As can be seen, all of the compounds showed non-linear van't Hoff behavior at temperatures below ca. 30°C, and linear behavior above ca. 30°C. Temperatures above ca. 38°C are recommended for good efficiency at an acceptable capacity factor. Linear temperature dependence of retention for C-18 columns using hydro-organic mobile phases has been reported (34-36). Non-linear dependencies have also been

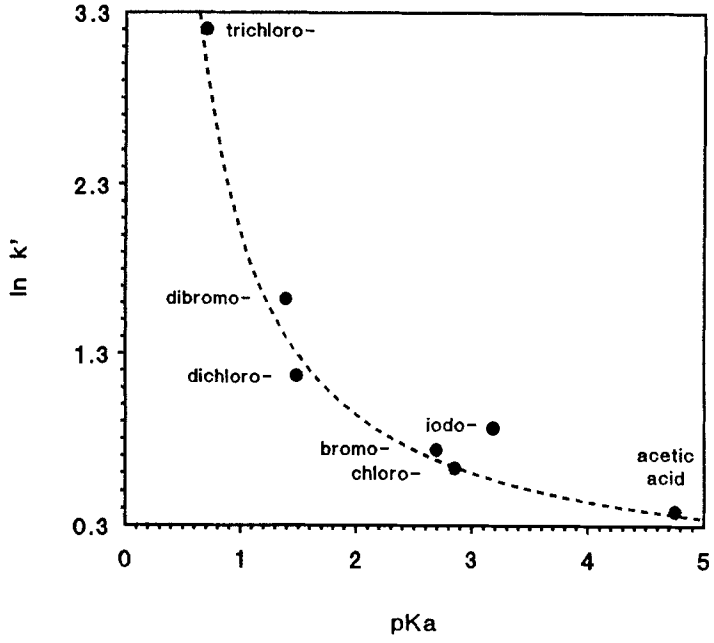

FIGURE 3 Plot of lnk' versus pKa for acetic acid and the following haloacetic acids: trichloro-, dibromo-, dichloro-, iodo-, bromo- and chloro-.

observed for gas chromatography and reversed phase liquid chromatography with silicas chemically bonded to n-octyl, n-octadecyl, aminobutyl and 2,4-dinitroanilinobutyl groups (37-39). A debate has arisen about the nature of phase transitions in these kind of stationary phases. Transitions can occur due to conformational changes in the stationary phase or the solute or due to changes in the ionization of the acid studied. It is unlikely that the solutes studied here would undergo conformational changes under the conditions studied, and the pH of the mobile phase used (5.2) should be high enough to keep the acids (with the exception of acetic acid) in their ionic form. Therefore, it is reasonable to assume that a real phase transition, with a corresponding change in the retention mechanism is occurring.

Our 30°C transition temperature is similar to that reported by other workers. Transitions have been reported for monomeric C-18 stationary phases at

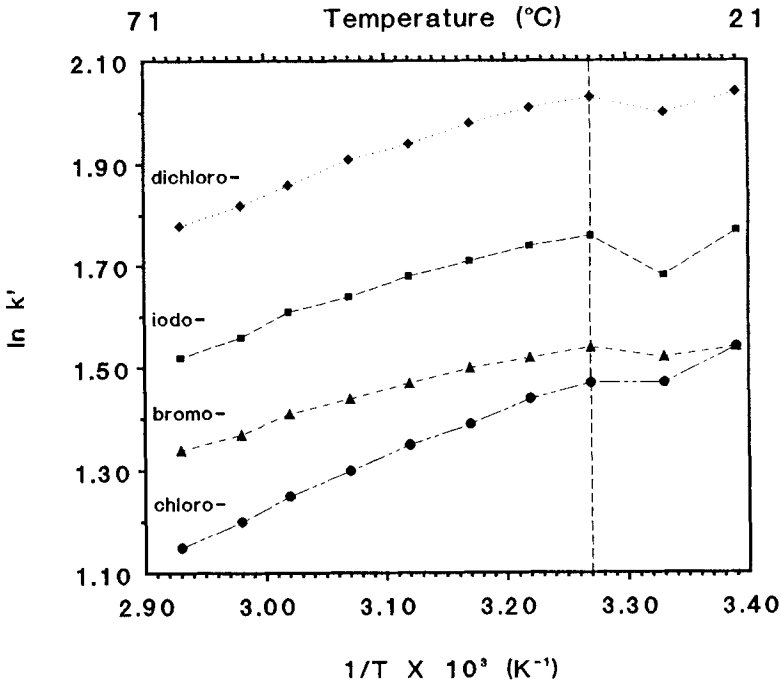

FIGURE 4 Plot of lnk' versus 1/T for chloro-, bromo-, iodo-, and dichloroacetic acid.

ca. 22°C in the absence of solvent using a gas chromatographic technique (40,41) and ca. 30-31°C for monomeric reversed-phase HPLC stationary phases (35,42). The present results indicate that below the phase transition point (30°C) the stationary phase may behave more like a solid (governed by surface adsorption), likely resulting in decreased interaction between the relatively bulky benzyltributyl-ammonium ion and the stationary phase, thereby reducing retention. Above the phase transition point, the stationary phase behaves more like a liquid allowing partitioning and deeper penetration of the ion pair reagent into the stationary phase and therefore increased retention, supported by workers who report a vertical penetration of the solute molecules into the bonded layer (43-45).

TABLE 3

Logarithm of the Capacity Factors Obtained at Different Temperatures. Detector = 260 nm, Flow rate = 0.6 ml/min, [KH_2PO_4] = 5.66 mM, [1-hexanesulfonate] = 0.13 mM, [acetonitrile] = 11%, pH = 5.2, [benzyltributylammonium] = 8.22 mM.

Temp. (°C)	lnk' for -acetic acid				
	Chloro-	Bromo-	Iodo-	Dichloro-	Dibromo-
22.1	1.545	1.545	1.775	2.039	2.686
26.4	1.470	1.515	1.677	2.004	2.642
30.0	1.470	1.541	1.756	2.028	2.664
33.7	1.437	1.524	1.740	2.017	2.626
38.5	1.391	1.500	1.712	1.981	2.590
42.5	1.348	1.475	1.681	1.944	2.542
46.5	1.302	1.442	1.645	1.905	2.480
50.6	1.250	1.409	1.607	1.864	2.425
54.9	1.203	1.374	1.564	1.820	2.365
58.1	1.154	1.338	1.522	1.775	2.303

Effect of phosphate concentration

Figure 5 illustrates the dependence of the lnk' of acetic acid, chloro-, iodo-, and dichloroacetic acid on the monobasic potassium phosphate concentration (complete data in Table 4) employing a Spherisorb C18 column. The capacity factor values of the solutes decrease as the phosphate concentration increases, in agreement with other studies investigating the influence of eluent ionic strength on retention in ion-pair HPLC systems (19,27,28). Using the ion-interaction model, retention begins with the establishment of an equilibrium between the lipophilic reagent ion (ion interaction reagent) and the stationary phase followed by retention of a sample of opposite charge to the reagent ion depending on both electrostatic attraction and a lipophilic adsorptive force. Therefore, increasing ionic strength should decrease retention by influencing the electrostatic attraction (27). We have

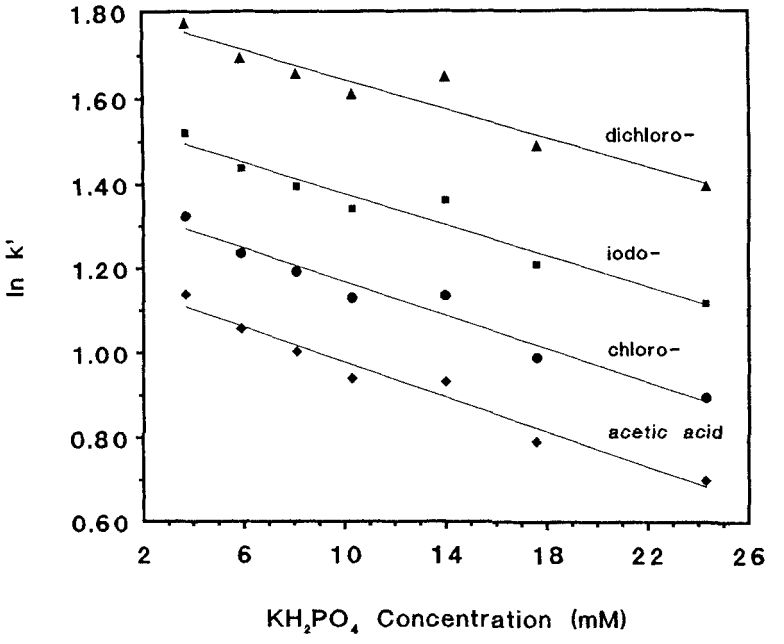

FIGURE 5 lnk' as a function of KH_2PO_4 concentration for acetic acid, chloro-, iodo- and dichloroacetic acid.

also observed that response is increased and peaks shapes are improved at higher concentrations of monobasic potassium phosphate.

Effect of hexane sulfonate concentration

Increasing the concentration of the hexane sulfonate in the mobile phase results in a corresponding decrease in retention for the acids as seen in Table 5 using a Spherisorb C18 column. Figure 6 illustrates the lnk' values of three of the acids as a function of the hexanesulfonate concentration (the effect is similar for all of the acids). Again, this is the expected behavior, given that the hexane sulfonate ions, being of like charge to the solutes, will compete for the ion interaction reagent and decrease the retention of the solutes. There is almost no variation of the selectivity when the concentration of the hexane sulfonate varies. These results

TABLE 4

Logarithm of the Capacity Factors Obtained With Different Concentrations of KH_2PO_4. Flow rate = 0.7 ml/min, temperature = 52.3°C, [1-hexanesulfonate] = 0.13 mM, [acetonitrile] = 11%, pH = 5.2, [benzyltributylammonium] = 8.22 mM.

[KH2PO4]	lnk' for acid					
	Acetic	Cl-	Br-	I-	DCl-	DBr-
3.7	1.138	1.323	1.372	1.520	1.774	2.296
5.9	1.057	1.236	1.295	1.439	1.695	2.227
8.1	1.004	1.193	1.248	1.396	1.659	2.193
10.3	0.941	1.131	1.186	1.342	1.612	2.146
14.0	0.933	1.136	1.193	1.362	1.651	2.200
17.6	0.791	0.989	1.057	1.208	1.490	2.041
24.3	0.700	0.894	0.959	1.115	1.393	1.957

TABLE 5

Logarithm of the Capacity Factors Obtained With Different Concentrations of 1-Hexanesulfonate. Flow rate = 0.8 ml/min, temperature = 51.4°C, [KH_2PO_4] = 5.66, [acetonitrile] = 11%, pH = 5.3, [BTA] = 8.22 mM.

[1-hexane-sulfonate]	lnk' for acid					
	Acetic	Cl-	Br-	I-	DCl-	DBr-
0.05	0.858	1.045	1.078	1.241	1.505	1.982
0.10	0.803	0.983	1.025	1.179	1.438	1.908
0.13	0.852	0.934	0.988	1.128	1.384	1.848
0.16	0.713	0.888	0.932	1.076	1.333	1.793
0.19	0.673	0.841	0.886	1.029	1.280	1.737

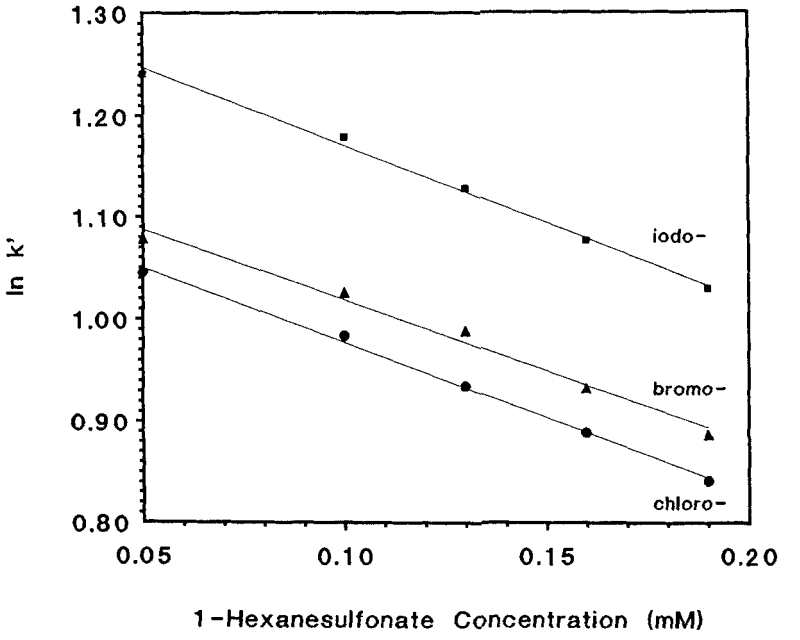

FIGURE 6 lnk' as a function of 1-hexanesulfonate concentration for chloro-, bromo- and iodoacetic acid.

are different than those reported previously for inorganic anions in a similar chromatographic system, where significant variations in selectivity were observed even with small variations in hexane sulfonate concentration (28). In addition to reducing the retention time of the solutes, in the present study, increasing hexane sulfonate concentrations increased efficiency and improved peak symmetry.

Effect of acetonitrile concentration

The effect of acetonitrile concentration on the capacity factors of acetic, chloroacetic, bromoacetic, iodoacetic, dichloroacetic and dibromoacetic acids are shown in Figure 7 (From data in Table 6) employing a Spherisorb C18 column. As expected, the acetonitrile concentration produces a dramatic effect on the retention of all of the solutes. Hydrophobic effects are known to dominate in the

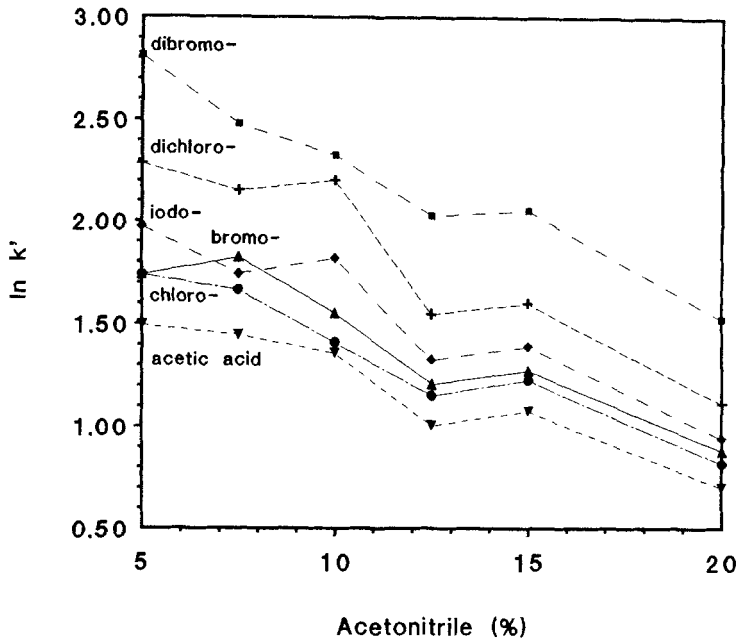

FIGURE 7 lnk' as a function of the percent (v/v) acetonitrile for acetic acid, chloro-, bromo-, iodo-, dichloro- and dibromoacetic acid.

water/organic solvent binary eluent systems commonly employed in RP-IPC (46). Hydrophobic expulsion is generally attenuated by increasing the concentration of organic modifier. Although some selectivity variations are seen at acetonitrile concentrations below ca. 10%, these separations generally yielded broader, less symmetric peaks and poorer reproducibility compared to separations performed at 10% acetonitrile and above. Previous workers have demonstrated linear relationships between the capacity factor of analytes and the ion interaction reagent for various IPC systems (21,33,47).

Effect of the mobile phase pH

Table 7 lists the results obtained when mobile phases are adjusted to different pH values. No separation was observed at pH below 3, and very little

TABLE 6

Logarithm of the Capacity Factors Obtained With Different Concentrations of Acetonitrile. Flow rate = 0.8 ml/min, temperature = 52.2°C, [KH_2PO_4] = 3.68 mM, [1-hexanesulfonate] = 0.13 mM, pH = 5.2, [BTA] = 8.22 mM.

[acetonitrile]	lnk' for acid					
	Acetic	Cl-	Br-	I-	DCl-	DBr-
5.7	1.495	1.739	1.739	1.975	2.287	2.814
7.5	1.448	1.665	1.824	1.743	2.152	2.481
10.0	1.361	1.414	1.554	1.820	2.204	2.329
12.5	1.007	1.153	1.210	1.328	1.549	2.028
15.0	1.078	1.229	1.276	1.393	1.603	2.055
20.0	0.701	0.819	0.881	0.944	1.115	1.526

separation was seen at pH above 7. Reasonable separation was achieved from pH 3.5 to pH 6.3. Figure 8 is a plot of relative capacity factor (k'/k'max) versus pH, (k'max is the maximum capacity factor). The dependence on the pH on retention for the present system is in agreement with results reported by other workers, where, at low pH, the acids are no longer ionized, and, at high pH, hydroxyl ions formed compete with the sample anions leading to a smaller number of sample ion pairs in the stationary phase (48). For the acids in the present study, a pH of ca. 5 was found to be optimal.

Effect of ion interaction reagent concentration

Results for the effect of benzyltributylammonium ion concentration on retention are summarized in Table 8. No separation was obtained with concentrations below 6 mM. At 8 mM, all solutes, with the exception of acetic acid, was achieved. At 14 mM and above, the response of the UV detector used

TABLE 7

Capacity factors obtained with mobile phases adjusted to different pH values. Flow rate = 0.8 ml/min, temperature = 51.5°C, [KH_2PO_4] = 6.0 mM, [1-hexanesulfonate] = 0.13 mM, [acetonitrile] = 12%, [benzyltributylammonium] = 8.22 mM, and stationary phase = C18 Spherisorb.

pH	k' for acid					
	Acetic	Cl-	Br-	I-	DCl-	DBr-
2.41	0.00	0.00	0.00	0.00	0.00	0.00
3.47	2.01	2.41	2.60	3.00	3.76	7.10
4.34	2.09	2.65	2.65	3.27	4.29	7.19
4.58	2.59	3.19	3.19	3.79	4.84	7.82
5 46	2 77	3 33	3.58	4.15	5.50	9.50
6.33	1.65	1.83	2.20	2.36	3.21	6.20
7 26	1.95	1 95	1.95	1.95	2.59	5.09
8.31	1.82	1.82	1.82	1.82	2.28	4.80

(at 262 nm) was off scale. Therefore, for the present study, the useful range was determined to be between the limited range of 10 to 12 mM. Although the data set is too small to generalize any conclusions, the increase in capacity factors with increased ion interaction reagent concentration is in keeping with results reported previously by other workers (20).

Role of the stationary phase

The different analytical columns were compared by injecting the same sample composition under identical instrumental conditions as follows: column temperature = 38°C, flow rate = 0.8 ml/minute, mobile phase containing 12 mM benzyltributylammonium, 0.1 mM hexanesulfonate, 6 mM monobasic potassium phosphate, pH 5.2 and 16 % (v/v) acetonitrile. The Octyl (C-8) and Nucleosil C-4

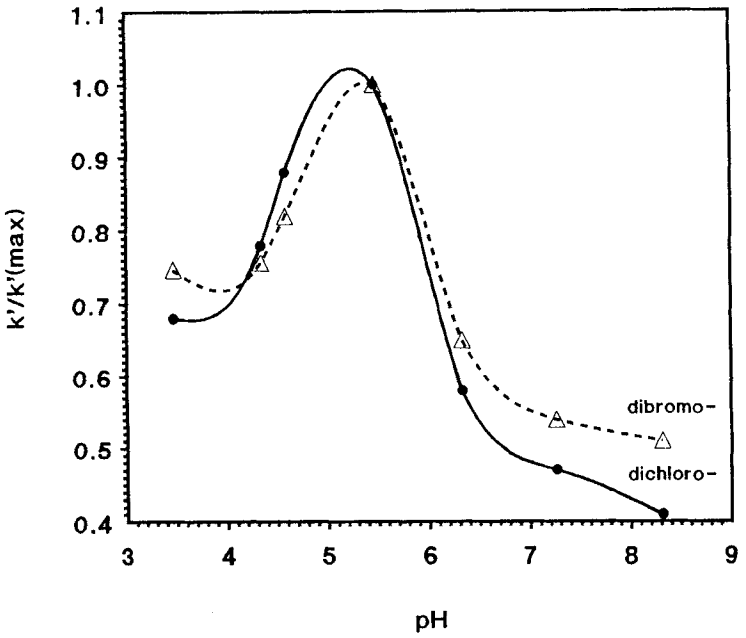

FIGURE 8 Plot of k'/k'(max) as a function of pH of the mobile phase for dibromo- and dichloroacetic acid.

TABLE 8

Capacity Factors Obtained With Mobile Phases Containing Different Concentrations of Benzyltributylammonium Ion. Flow rate = 0.8 ml/min, temperature = 51.5°C, [KH_2PO_4] = 60 mM, [1-hexanesulfonate] = 0.13 mM, [acetonitrile] = 12%, pH = 5.45, and stationary phase = C18 Spherisorb.

[BTA] (mM)	k' for acid					
	Acetic	Cl-	Br-	I-	DCl-	DBr-
6	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	1.66	1.78	2.06	2.74	4.55
10	1.45	1.75	1.90	2.20	2.92	4.94
12	1.47	1.88	2.09	2.38	3.23	5.08
14	----------MOBILE PHASE OFF SCALE----------					

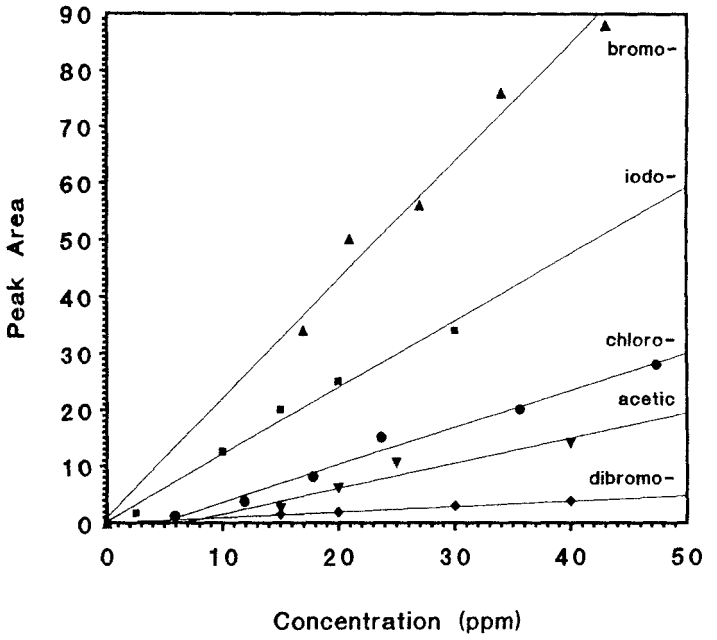

FIGURE 9 Peak areas obtained at 262 nm with different concentrations of acids. Flow rate = 0.6 ml/min, temp. = 38.0°C, [KH2PO4] = 5.66 mM, [1-hexanesulfonate] = 0.13 mM, [acetonitrile] = 11%, pH = 5.2, [BTA] = 8.22 mM, and stationary phase = Spherisorb C18.

yielded the poorest separation, able to separate just a few of the acids. The phenyl column showed very good separation for all compounds (except acetic acid) and very good peak shapes, but capacity factors for the different acids were low, which could be a problem if a more complex sample, containing other compounds with retention times in the same range, were to be analyzed. Hypersil C-18, demonstrated excellent separation, but poor peak shapes for dichloroacetic and dibromoacetic acids. Overall, the best results were obtained for Spherisorb C-18 and Nucleosil C-18, with convenient capacity factors and good peak shapes.

Calibration curves and detection limits

The linearity and the detection limit of the system was evaluated for all of the acids using UV absorption at 262 nm. The mobile phase contained 8.22 mM

benzyltributylammonium, 0.13 mM hexanesulfonate, 5.66 mM monobasic potassium phosphate, pH 5.2 and 11 per cent acetonitrile in water. The flow rate was 0.6 mL/minute at a column temperature of 38°C. All acids were determined in the presence of the other five, in concentrations ranging from 2 to 48 ppm with linear responses seen for all of the acids as seen in Figure 9. The detector response and detection limits were highly dependent on the acid with better sensitivity for the monohaloacetic acids than for the dihaloacetic acids. Detection limits were estimated by determining the lowest concentration which yielded significant and reproducible detector response with a signal-to-noise ratio of ca. 4. Detection limits were in the range 2-15 ppm with correlation coefficients listed below: Acetic acid = 15 ppm (r = 0.949); Chloroacetic acid = 2 ppm (r = 0.993); Bromoacetic acid = 8 ppm (r = 0.994); Iodoacetic acid = 3 ppm (r = 0.994); Dichloroacetic acid = 10 ppm (r = 0.993); Dibromoacetic acid = 15 ppm (r = 0.998).

CONCLUSIONS

Of the commercial columns tested, Spherisorb C-18, yielded the best results for the separation of the haloacetic acids studied. This column exhibited a linear van't Hoff behavior (decrease in lnk' with increasing temperature) in the range of ca. 30°C to 60°C with a phase transition resulting in a loss of resolution below 30°C. The capacity factor of the acids decreases with increases in the phosphate concentration and increases in the concentration of 1-hexanesulfonate in the mobile phase with little variation in the selectivity. Values of pH from 3.5 to 6.3 provided acceptable resolution with optimal conditions seen at ca. pH 5. Retention of the analytes increases with increasing benzyltributylammonium ion with an optimal value between 10 and 12 mM. Increasing the acetonitrile concentration produces one of the most significant effects (in the usable concentration range), resulting in a significant decrease in the retention of the acids studied.

The relative effects of the six variables studied can be compared by processing all of the data for one of the solutes, chloroacetic acid, and calculating the theoretical change which would be required to decrease the k' by one unit (i.e. from 4 to 3), corresponding to a decrease in the lnk' of 0.3 (i.e. from 1.4 to 1.1). This change would require increasing the column temperature by 27°C, increasing the KH_2PO_4 concentration by 15 mM, increasing the 1-hexanesulfonate

concentration by 0.2 mM, decreasing the pH by 2 units (depending on the starting pH, i.e. from 5.5 to 3.5), decreasing the benzyltributylammonium concentration by 10 mM (although this would likely be below the minimum of 8 mM depending on the starting point), or increasing the amount of acetonitrile by 5 % (v/v).

This chromatographic method is able to separate a mixture of acetic, chloroacetic, bromoacetic, iodoacetic, dichloroacetic, and dibromoacetic acids with good resolution in less than 20 minutes under isocratic conditions. Aqueous samples can be injected directly, with no sample preparation and the system has a linear response for all of the acids in the range studied (up to 50 ppm). Detection limits as low as 2 parts per million were possible using a UV-visible detector and lower detection limits may be feasible utilizing indirect fluorescence detection as the UV-absorbing mobile phase employed here is also intensely fluorescent.

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